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Ontogeny of Adrenergic Arousal and Cholinergic Inhibitory Mechanisms in the Rat

Abstract. *With spontaneous activity as a measure of arousal, dose response curves were established for scopolamine and amphetamine administered to 10-, 15-, 20-, 25-, and 100-day-old rats. Amphetamine always increased activity, but scopolamine had no effect on younger rats, which suggests that adrenergic excitatory areas in the brainstem mature more rapidly than cholinergic inhibitory areas in the forebrain.*

Generalized excitatory and inhibitory systems in the brain regulate overall levels of arousal. The major excitatory center is thought to be the brainstem reticular formation. When activity in this area is destroyed by lesions, stupor results (*1*); when the area is activated by electrical stimulation, electrocortical and behavioral arousal occurs (*2*). Acting in opposition to this excitatory region are certain forebrain structures which serve to modulate reticular excitability. When these centers or their connections with the brainstem are impaired, the effects of reticular stimulants are greatly augmented (*3*). When this area is activated by electrical stimulation, arousal is depressed (*4*).

The biochemical substrates of the arousal areas in the hindbrain and inhibitory centers in the forebrain are distinct, with the former primarily adrenergic in nature and the latter predominantly cholinergic (*5*). Amphetamine, which mimics adrenergic transmission by release of norepinephrine (*6*), particularly in the brainstem (*7*), induces large increments in locomotor activity, while adrenolytic agents depress arousal and generally lead to sedation (*8*). The anticholinergic drug scopolamine, which blocks acetylcholine transmission by occupation of postsynaptic sites (*9*), produces marked increments in activity (*10*), while anticholinesterases (*11*) and cholinomimetic agents (*12*) depress arousal.

It is now generally accepted that the development of the brain proceeds rostrally with phylogenetically primitive hindbrain structures maturing earlier than the younger forebrain systems (*13*). Thus neonatal animals should pass through a phase during which they are responsive to reticular stimulants and unaffected by cholinergic blocking agents because of the functional absence of forebrain inhibitory mechanisms. We now show that the neonatal rat is responsive to the reticular stimulant amphetamine, before it is responsive to scopolamine, an inhibitor of forebrain cholinergic activity.

Degree of behavioral arousal was measured in stabilimeter activity cages scaled to the size of the animal. The largest cages, those used for the adults (*14*) consisted of wire mesh cages, 17.5 by 20.0 by 37.5 cm, mounted on a central axle which permitted the cage to tip slightly and activate a sensitive switch as the rat moved from one end to the other. For 10-day-old rats the cage dimensions were scaled down to 6.3 by 7.5 by 13.7 cm, and for 15-, 20-, and 25-day-old rats the cage was 8.7 by 10.0 by 18.7 cm. The activity cages were housed in temperature-controlled cubicles maintained at 29°C for the 10-, 15-, 20-, and 25-day-old rats and at 22°C for the 100-day-old rats.

Dose response curves for both amphetamine and scopolamine were determined at five different ages: 10, 15, 20, 25, and 100 days. At each dose 10 to 16 rats were tested, and each rat was tested only once. A total of 772 Sprague-Dawley rats were used, half of which were male and half female.

Rats were removed from living cages in a central colony room, placed in the activity cages for a 30-minute habituation period, and then injected with one dose of either *d*-amphetamine sulfate (0.250, 0.50, 1.0, 2.0, 4.0, or 8.0 mg/kg, salt weight), scopolamine hydrochloride (0.125, 0.250, 0.50, 1.0, 2.0, or 4.0 mg/kg, salt weight) or an equivalent volume of the 0.9 percent saline vehicle. They were then returned to the cages for 2 hours; during this time the number of crossings was recorded on printing counters every half hour. In addition, methylscopolamine hydrobromide (0.125, 0.250, 0.50, 1.0, 2.0 or 4.0 mg/kg, adjusted for equivalent amounts of scopolamine), a drug which does not cross the blood-brain barrier in significant quantities (*15*), was administered to a group of 25-day-old rats for control of the possible peripheral effects produced by the scopolamine hydrochloride. All drugs and the saline control were administered intraperitoneally in a volume of 1 ml per kilogram of body weight.

Figure 1 shows the mean amount of activity occurring during the entire 2-hour test period for all groups. Amphetamine produced an increment in activity, proportional to dosage, in animals at all of the ages studied, while scopolamine increased activity only in animals 20 days of age and older. Methylscopolamine had no systematic effect on activity, which indicates that the scopolamine-induced increase in activity was the result of central rather than peripheral effects. No consistent sex differences in response to the drugs were found except in the 100-day-old group, where the females were more active regardless of whether drugs were given or not.

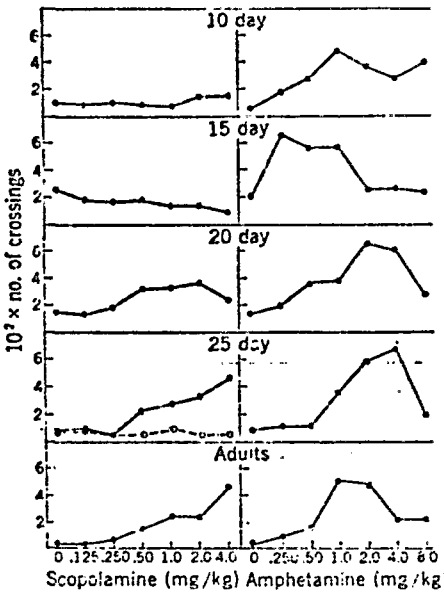

Fig. 1. The effects of scopolamine hydrochloride and *d*-amphetamine sulfate on spontaneous activity of rats of five different ages. The effect of methyl scopolamine is shown by the dotted line in the 25-day panel.

The results also suggest that the maximum effective dose of amphetamine was somewhat dependent on age. The 15-day group appeared to be more sensitive to amphetamine than the older animals insofar as they showed maximum activity increases in response to low dosages, whereas higher dosages were required to elicit maximum response in the older animals.

These results demonstrate that reactivity to amphetamine and scopolamine matures at different rates in the neonatal rat. To interpret these results, it is most plausible to assume that amphetamine acts by releasing endogenous norepinephrine in primitive hindbrain

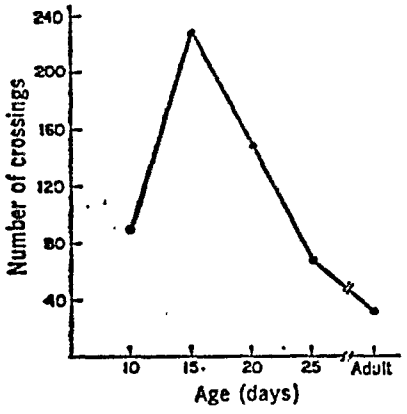

Fig. 2. Spontaneous activity as a function of age in nondrugged animals.

arousal centers and that these centers mature earlier than the forebrain inhibitory centers do. In turn, scopolamine cannot act to block the cholinergic forebrain inhibitory centers until these centers are functionally mature and exerting a chronic inhibitory influence on hindbrain activity.

Additional evidence for the delayed maturation of forebrain inhibitory centers is found in the activity pattern of the saline control groups. When these data (combined means for all saline control groups of the same age) are plotted separately so that the age-related trends are not masked by the larger drug effects (Fig. 2), it is apparent that spontaneous activity in a novel environment reaches a peak in rats at about 15 days of age and declines rapidly in the subsequent 10 days. The increase in activity between days 10 and 15 undoubtedly reflects increasing skeletal muscular development plus increasing sensory responsiveness (*16*). The decline in normal activity corresponds strikingly to the increasing effectiveness of scopolamine as a behaviorally arousing drug, which suggests that forebrain cholinergic inhibitory centers also act to modulate exploratory activity in novel environments.

Moreover, this period of declining arousal and increasing sensitivity to anticholinergics also parallels functional development of the forebrain. Primitive electroencephalographic activity is first noted at 6 days after birth, but the spectral composition does not approximate that of the adult until the rat is between 25 and 30 days of age (*17*). Myelin, which is present in the brainstem at birth, is not seen in the forebrain until 10 days after birth, with the greatest deposition occurring between 15 and 30 days of age (*18*). Similarly, the number of synaptic junctions in the cortex undergoes massive proliferation between 15 and 25 days of age (*19*).

Considerable evidence from studies of humans supports the view that the forebrain areas exert inhibitory control over hindbrain mechanisms (*20*). Both the human infant and the rat display little more than simple involuntary responses at birth. With maturation, many of these reflexes disappear and then reappear with corticol atrophy in senescence or after cortical injury (*21*). Forebrain development thus appears to modulate both primitive reflexes and behavioral arousal. Our data suggest that at least some portion of this inhibitory mechanism is cholinergic.

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